Spectroscopic Window on Tumor Metabolism

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Introduction

In vivo MRS can be performed along with MRI to measure metabolic properties of tumors. MRS has proven its value as a research tool for elucidating metabolic and biochemical aspects of cancer. In addition, *in vivo* MRS holds much promise for becoming an indispensable clinical tool for detecting, diagnosing, and monitoring responses to therapies in patients. Well known examples of the latter include the use of MRS to improve the accuracy in differentiating between malignant and benign lesions and to detect an early response to a chosen therapy. At present, a growing number of radiologists as well as oncologists are interested in incorporating MRS into their MRI protocols. This article introduces ^{1}H MRS of cancer, discusses current methods and technical issues, and describes some of the more common applications to cancer. Although *in vivo* MRS has been used to study cancers in many different parts of the body, the greatest progress in the development and validation of MRS has occurred mainly for three types of cancer: brain, breast, and prostate. Thus, the present discussion will focus mainly on these cancers, although promising results have been obtained in pilot ^{1}H MRS studies of cancers in other sites, including extracranial lymphoma and germ cell tumors [1], hepatic tumors [2], head and neck tumors [3], melanoma, and lymph nodes [4].

Altered ^{1}H metabolites in cancer

Malignant tissues have unique spectral features that distinguish them from normal tissues. Many of the metabolite resonances present in the normal host tissues are absent or decreased in malignant lesions. For example, resonances from polyamines and citrate (Cit), which are hallmarks of normal healthy and benign hyperplastic prostatic tissues, are absent or substantially reduced in prostate cancers. Likewise, resonances from N-acetylaspartate (NAA), a neuronal marker, are typically reduced in brain tumors. Total creatine (tCr), an indicator of bioenergetic status, is another resonance that typically has altered intensity in cancers. Other hallmarks of cancer include increased levels of choline-containing compounds (tCho), mobile lipids, and lactic acid. Due to their importance in cancer, each of these compounds is discussed in further detail below.

Choline compounds

Numerous *in vivo*, *ex vivo*, and *in vitro* studies have demonstrated elevated levels of tCho in neoplastic tissues and cells. *In vivo* ^{1}H spectra of cancerous lesions typically display a

prominent resonance from choline compounds at 3.2 ppm. *Ex vivo* studies have been performed to identify the different choline compounds giving rise to this resonance. In breast cancer, high-resolution ^{1}H spectra acquired from biopsy tissues have shown that the 3.2 ppm resonance is actually a superposition of several resonances [5-7]. The primary constituents are those with a trimethylamine moiety [$R\text{-}(CH_2)_2\text{-}N^+\text{-}(CH_3)_3$], including free choline (Cho), phosphocholine (PCho), and glycerophosphocholine (GPC). Other metabolites possibly contributing include taurine, glucose, phosphoethanolamine, and myo-inositol (mIns) [7]. The choline head groups associated with semi-mobile lipids may also contribute. These resonances can be separated in *ex vivo* studies with high-resolution NMR spectrometers, but *in vivo*, these peaks are substantially broadened, and at fields as high as 4 Tesla these resonances are generally indistinguishable. Consequently, the simplified approach used in *in vivo* studies is to treat the 3.2 ppm spectral peak as a single resonance, and thus it is referred to as tCho (total choline-containing compounds).

The precise mechanisms that produce elevated tCho levels in cancers are as yet not fully understood. A working hypothesis is that elevated tCho is an indicator of increased cellular proliferation [8, 9]. Indeed, the largest component contributing to the tCho peak from neoplastic tissue is usually PCho, a known precursor of membranes. Another major component, GPC, is a breakdown product of membranes. It is known that tCho levels can be modulated by numerous changes in enzymatic activity and fluxes in these anabolic and catabolic pathways [10, 11], and the increased tCho level in neoplastic tissues may reflect increased membrane turnover in neoplastic tissues. In the author's opinion, the present understanding of elevated tCho in malignant cells remains an oversimplified view, and further research is needed to obtain a complete picture of the biological processes leading to elevated tCho. It can be expected that MRS will likely play a vital role in elucidating these processes.

Lipids

Resonances from mobile lipids can be observed in ^{1}H spectra of breast [12], brain [13], and prostate tumors [14]. The amplitude of the lipid resonance can vary dramatically depending on the tissue heterogeneity. Lipid peaks in tumor spectra can arise from sources other than normal adipose tissue. For example, resonances from mobile lipids have been shown to occur in necrotic tissues [15]. Furthermore, experimental data from *in vitro* cell studies suggest the formation of intracellular lipid droplets are a source of lipid resonances [16]. In studies of T-cell lymphoblast cultures, a correlation between the methylene-to-methyl signal ratio and number of apoptotic cells was found [17]. In accordance with this finding, lipid levels were found to correlate with apoptosis and early cell death in rat BT4C gliomas [18]. The presence of elevated lipid resonances in human astrocytic tumors has been suggested to have prognostic significance [19].

In human breast, the adipose tissue which is not directly involved in the carcinoma can pose significant problems for *in vivo* ^{1}H MRS. When trying to choose the volume-of-interest (VOI) for localized MRS studies of breast cancer, any adipose tissue inadvertently included in the VOI creates a partial volume effect, reducing the effective volume for spectroscopy. Likewise, in studies of prostate and brain tumors, it is very important to suppress intense signals from adipose tissues which surround the prostate and brain, in order to avoid spectral contamination ("bleed effect") and artifacts (e.g., baseline distortion). Adipose tissue also limits the ability to optimize (or "shim") the homogeneity of the magnetic field inside the VOI, which in turn leads to broad resonances and reduced signal-to-noise ratio. Intense lipid resonances can also produce sideband artifacts which can interfere with MRS measurements [20]. These artifactual resonances can be larger than the tCho resonance. To reduce sideband artifacts, our

group uses a method called echo-time (TE) averaging, which causes coherent cancellation of sideband artifacts by averaging spectra acquired at several different TE values [20].

Lactate

Under hypoxic conditions, neoplastic cells are thought to derive energy primarily from glycolysis. Even in the presence of oxygen, malignant lesions can have an increased glycolytic metabolism. Many investigations of experimental tumors have provided evidence of reduced respiratory capacity and increased reliance on glycolysis for energy production. Consequently, the concentration of lactate in tumors is generally higher than that in normal tissues. In single-voxel ^{1}H MRS measurements on normal human brain the lactate concentration was ~0.6 mmol/kg [21], whereas in untreated human brain tumors the lactate levels were found to be 2.6 ± 0.8 mmol/kg (mean ± S.D., n=7) [22]. In studies of rat C6 glioma, we observed a positive correlation between the tumor lactate levels measured by *in vivo* ^{1}H MRS and the neoplastic cell density determined by histopathology [23]. Of interest, *ex vivo* studies (non-MRS) have revealed a positive correlation between the incidence of metastasis and the mean lactate concentration in biopsy specimens of cervical and head and neck tumors [24, 25].

Applications

Breast Cancer

The first *in vivo* MRS studies of breast measured resonances from ^{31}P nuclei. These studies showed that measurable variations in phospholipid metabolism could be detected and used for diagnosing cancer and monitoring response to treatment (reviewed in [26, 27]). More recently, there has been growing interest in breast cancer research using ^{1}H MRS, due to its higher sensitivity than ^{31}P MRS. The first breast ^{1}H MRS reports focused on the diagnostic utility of the water:fat ratio in the breast [28-30], but subsequent studies did not find this ratio to be a useful diagnostic metric [31, 32]. However, a number of studies performed with ^{1}H MRS noted the presence of tCho in spectra of malignant lesions, but not in benign or normal tissues [29, 31-36].

The majority of breast MRS studies to date have used single-voxel spectroscopy (SVS) to localize the spectrum to a single volume centered on the lesion of interest. While most breast MRS studies have been done with SVS, other researchers have explored the use of spectroscopic imaging (MRSI) as an alternative [37]. With MRSI, a grid of spectra are acquired. MRSI has an important advantage – it provides information about the spatial distribution of metabolites, which is useful for studying multiple lesions or evaluating the spatial variation of a metabolite in a heterogeneous lesion. However, MRSI for breast is technically more challenging than SVS and quantification of metabolite levels is more problematic. For these reasons, most MRS research on breast cancer has used SVS. Figure 1 shows a representative example of a single-voxel ^{1}H spectrum of an invasive ductal carcinoma, with the tCho resonance indicated.

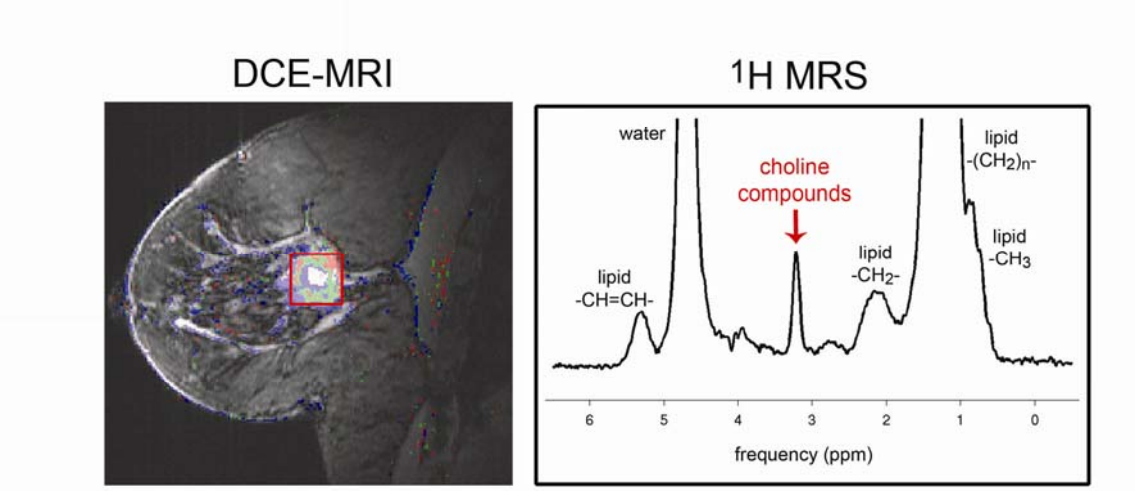

Figure 1 - Example of a localized breast ^{1}H spectrum of human breast acquired at 4 Tesla. The fat-suppressed, dynamic contrast-enhanced MR image (sagittal view) on the left shows the location of the VOI (red box), which covers a rim-enhancing invasive ductal carcinoma. The water-suppressed spectrum on the right shows the resonances observed in this malignant breast lesion.

While a number of groups have had success using the *detectability* of tCho to indicate malignancy, this approach assumes that the MRS measurement sensitivity is roughly constant from one measurement to the next. However there are a number of factors which make this assumption questionable in breast MRS, such as variations in voxel size, adipose tissue content, and coil sensitivity. In our experience at 4 Tesla, we have found that these factors produce a large variation in sensitivity [38]. Thus, some form of quantification should be used to correct for sensitivity variations, or at the very least exclude those voxels with unusual sensitivity.

A variety of approaches have been used for quantifying MRS data. We chose to use the intravoxel water resonance as an internal reference, because this method is robust and automatically compensates for variations in many factors [38]. Some groups have proposed using an external standard for referencing [31, 39]. This approach also works, but requires additional corrections for voxel size, adipose tissue content, and coil efficiency. Both internal and external referencing methods need correction for differences in relaxation rates which are difficult to measure in individual subjects.

A typical breast MRS study is performed immediately after acquiring dynamic contrast-enhanced (DCE) MR images. Decisions about the placement of the MRS voxel are usually based on review of the lesion morphology and the kinetics of contrast agent uptake while the patient is still in the magnet. With SVS, the placement of the voxel is of critical importance. The voxel should be placed so that it contains as much of the lesion as possible while excluding other tissues such as normal fibroglandular or adipose tissue. In studies using quantitative ^{1}H MRS to monitor response to treatment, the voxel size and position can be adjusted to cover the same anatomical region of the tumor and the voxel size is decreased as the tumor shrinks.

The first and most studied application for breast MRS is to distinguish benign from malignant lesions prior to biopsy. The first published paper on this topic, by Roebuck *et al*. in 1998 [31], proposed the idea that tCho could be used as a marker of malignancy. A number of papers that followed continued to use this hypothesis, but performed studies with somewhat different techniques. The overall results are quite consistent. Katz-Brull *et al*. published a combined analysis of the first five publications and reported an overall sensitivity of 83% and

specificity of 85% [40]. These results are very encouraging, especially considering that the determination of malignancy was done without considering any other diagnostic or historical information that would normally be available clinically.

Other publications describing tCho detection in breast cancers did not report diagnostic specificity and sensitivity [30, 32]. While the results using a simple detectability hypothesis are encouraging, it seems likely that there are benign pathologies that also produce detectable levels of tCho. Indeed, at 1.5 Tesla, a detectable tCho resonance has been reported in fibroadenomas [33, 34, 37, 41], tubular adenomas [31, 35], and lactating subjects [33, 36].

Two recent studies have aimed to evaluate whether MRS can improve the specificity of a diagnostic breast MR exam. Huang *et al*. appended a single-voxel MRS measurement and a single-slice T_2^*-weighted perfusion measurement to a conventional DCE-MRI exam [41]. They found that the addition of MRS increased the specificity of the exam from 62.5% to 87.5%, and the further addition of the perfusion measurement raised the specificity to 100%. Our group recently performed a retrospective blinded observer performance study with four readers and 55 subjects to determine if quantitative MRS could improve the specificity and sensitivity of a DCE-MRI exam [42]. In this study, we reported that adding quantitative MRS results to a DCE MRI exam produced improvements in the sensitivity, specificity, and accuracy for all readers, and improved the inter-observer agreement between the readers.

A second promising application of breast MRS involves predicting response to treatment. Current clinical methods such as palpation and imaging rely on changes in tumor size, which typically take several weeks before any changes are detectable. Breast MRS, in contrast, detects changes in intracellular metabolism that would occur before any gross morphological change. The first report using tCho measurements to detect treatment response in breast cancer was by Jaganathan *et al*., who observed that the tCho resonance disappeared or became smaller in 89% of subjects undergoing chemotherapy [36]. Expanding on this observation, our group performed a study designed to determine whether changes in tCho concentration ([tCho]) could provide a biomarker of clinical response as soon as 24 hours after the first dose of doxorubicin-based chemotherapy for locally-advanced breast cancer [43]. Of the first 13 patients who successfully completed the protocol without technical problems, the change in [tCho] between baseline and 24 hours after the first dose of chemotherapy showed significant positive correlation ($R=0.79$, $p=0.001$) with the change in lesion size measured at the end of four cycles of chemotherapy. The change in [tCho] within 24 hours was significantly different between responders and nonresponders ($p=0.007$) classified using RECIST (Response Evaluation Criteria in Solid Tumors). These results suggest that the change in [tCho] within 24 hours after the first dose of the drug can serve as an early indicator for predicting clinical response to treatment for locally-advanced breast cancer.

Prostate Cancer

Unlike in breast applications, the suspicious regions in the prostate cannot be as easily localized with imaging and are typically multi-focal, necessitating the use of spectroscopic imaging instead of single voxel methods. In order to cover the entire prostate while maximizing resolution and signal-to-noise, three-dimensional ^{1}H MRSI is the method of choice. To localize the volume of interest while minimizing chemical shift effects, point resolved spectroscopy (PRESS) is used in combination with high bandwidth outer-volume suppression (OVS) [44]. Minimizing the effects of periprostatic lipids is accomplished with spatially and spectrally

selective RF pulses [45] or with the inclusion of spectrally selective suppression RF pulses [46-48].

Cancer in the prostate is characterized by increased tCho and decreased Cit levels. The decrease in citrate results from both a change in normal epithelial cell function and loss of ductal structure, while increased tCho levels, although not fully understood, may be due to increased cellular proliferation and density. Thus the ratio tCho/Cit is doubly sensitive to the abnormal metabolism, cellular function and morphology observed in cancer. As it is difficult in many instances to separate the resonances of tCho and tCr the ratio is typically reported as (tCho+tCr)/Cit.

Metabolic information provided by spectroscopic imaging has shown promise in targeting biopsy [49], following response to therapy [50], treatment planning [51, 52] and cancer staging [53]. Zakian et al has recommended using the combination of the maximum ratio (tCho + tCr)/Cit and the MR spectroscopic imaging tumor volume as an index to help predict tumor aggressiveness [54].

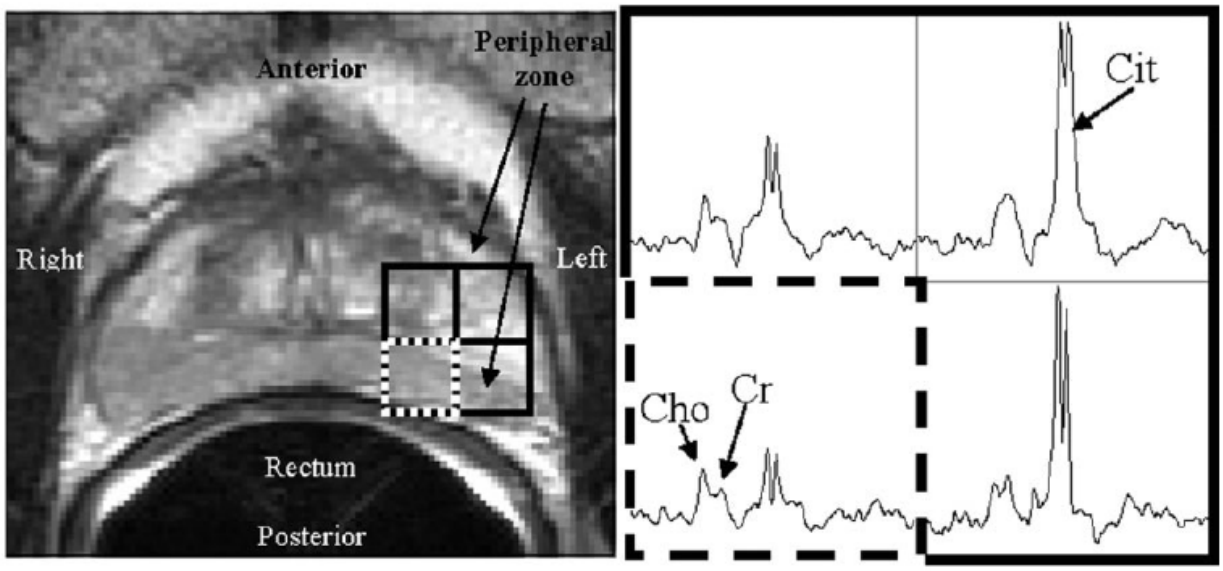

Figure 2 – T_2-weighted axial MRI (left) and selected ^{1}H spectra (right) from a patient with prostate cancer (Gleason score 6). The anterior part of the left peripheral zone has normal spectra (solid line) and is bright on the MR image, while the posterior par has cancerous spectra (dotted line). Reproduced from Noworolski et al, *Magn. Reson. Med. 53*:249-255 (2005).

Brain Tumors

Several early studies have suggested a role for *in vivo* ^{1}H MRS in helping to diagnose and monitor treatment response in brain tumors [55-60]. More recent publications have convincingly shown the value of ^{1}H MRSI in the clinical management of brain tumor patients in a number of areas. These include characterizing and classifying tumors [61], identifying tumor in T_2-hyperintense regions that did not display enhancement with Gd-contrast injection [62], differentiating cerebral necrosis from tumor progression [63, 64], monitoring treatment response [65-68], and predicting prognosis [69]. Similar to the prostate, 3D ^{1}H MRSI is generally considered to be the method of choice for evaluating brain tumors.

As compared to normal brain, common distinguishing spectral features of brain tumors include decreased levels of NAA and tCr, and elevated levels of tCho, lactate and lipids. The level of mIns, an astrocytic marker and putative osmolyte, has been reported to be elevated in hemangiopericytomas [70].

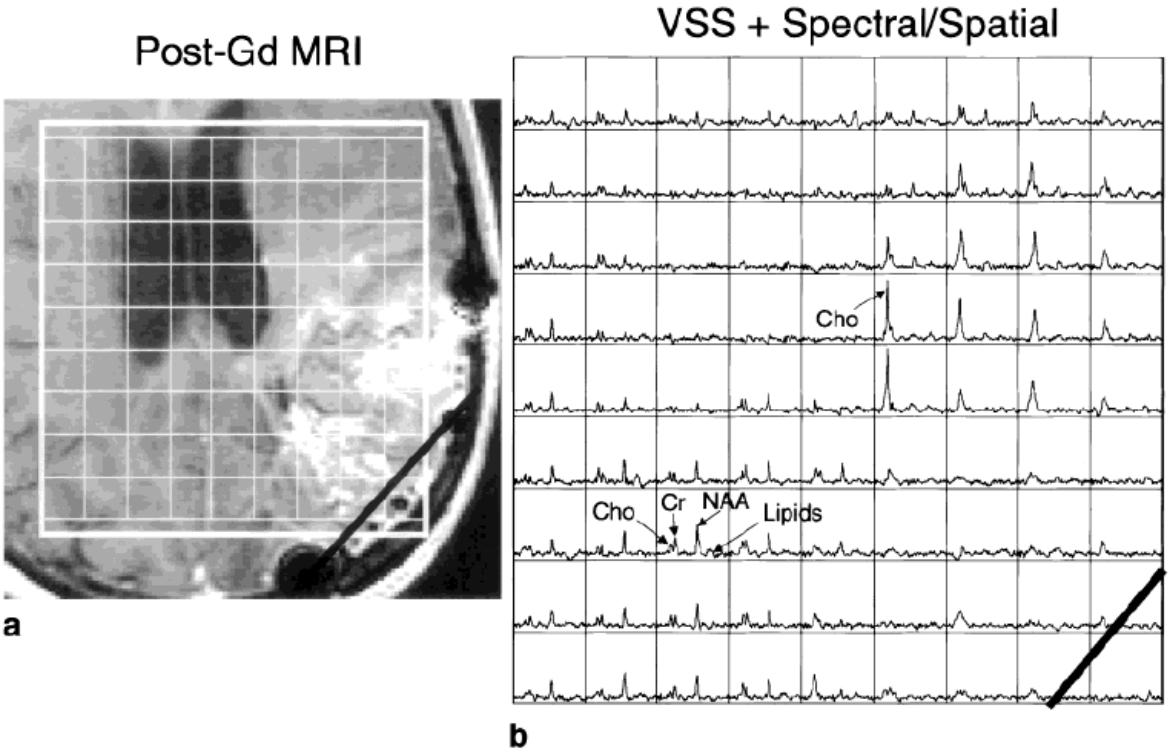

Figure 3 – Spoiled gradient-echo image (left) showing the placement of the PRESS-selected box and the MRSI grid used to obtain full coverage of the brain tumor mass. The PRESS-selected box includes the subcutaneous lipid layer at the bottom-right corner. By chopping the corner of the PRESS box using very selective OVS pulses (black line indicates edge of suppression band), contamination from mobile lipid signals arising in the scalp were avoided. Elevated tCho levels occurred in the viable tumor regions, while the absence of metabolites in other regions was indicative of necrosis. Reproduced from Tran et al, *Magn. Reson. Med. 43*:23-33 (2000).

Conclusions

The quality and reliability of MRS data will only improve as 3 Tesla systems become more common and as further refinements in techniques and software occur. Based on the convincing results obtained from multiple institutions to date, it is clear that ^{1}H MRS is destined to play a prominent role in the clinical management of cancer patients.

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